DNA, and protein from a single sample, said method comprising

collecting cells from a patient wherein the cell sample is limited in size;

storing collected cells in the medium according to claim 20;

removing an aliquot of cells in the medium for cell morphology analysis; and

removing a second aliquot of cells in the medium for a quantitative analysis selected from the group consisting of DNA analysis, RNA analysis, protein analysis and carbohydrate analysis.

REMARKS

The amendment to claim 1 simply removes the parenthetical phrase. The scope of the claim remains unaffected. The amendments to claims 5, 15, 16, 17, 20 and 24 simply insert commas as indicated by the Examiner, and these amendments do not alter the scope of the claims. The amendment to claim 18 simple changes the dependency of the claim. The amendment adds no new matter to the specification. Therefore, entry of the amendment is respectfully requested.

RESPONSE TO "PRIORITY"

Applicants have amended the specification to claim priority as specified in 37 C.F.R. §1.78.

RESPONSE TO "INFORMATION DISCLOSURE STATEMENT"

The information disclosure statement allegedly fails to comply with the Rules. In particular, DE 44 45 769 C1 is claimed to not be present. Applicants provide herewith a courtesy copy of DE 44 45 769 C1 and corresponding English language patent, U.S. Patent No. 5,786,337 which claims priority to DE 44 45 769.3. Applicants request an initialed copy of PTO FORM 1449 indicating the Examiner's review of the German Patent. In addition, applicants

provide a copy of a stamped Return Receipt Postcard indicating that the Patent Office received the three references cited in FORM PTO 1449, one of which is DE 44 45 769 C1.

RESPONSE TO CLAIM OBJECTIONS

Applicants have amended claims 5, 15, 16, 17, 20 and 24 as indicated by the Examiner. Reconsideration and withdrawal of the claim objections is, therefore, respectfully requested.

RESPONSE TO SECTION 112 REJECTION

Claims 2-4, 21 and 25-27 stand rejected under 35 U.S.C. §112, second paragraph, for being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In particular, the Examiner contends than the recitation "less than X ml" is confusing because it is not clear what the lower limit of volume is, i.e., that 0 ml may be encompassed by the claim. Applicants respectfully disagree with this rejection.

Claims 2-4 describe a medium for sample collection that allows for direct analysis by both cytological and molecular methods, claim 21 describes an article of manufacture for preserving a cell sample, and claims 25-27 describe a method of sample collection that allows both detection of cell morphology and quantitative analysis of a biopolymer. Thus, there can be no doubt as to the presence of medium, as without medium there would be no analysis or preservation of the sample possible. One skilled in the art would readily appreciate the minimum amount of medium necessary to analyze or preserve any particular sample. Therefore, in light of the arguments presented above, applicants respectfully request reconsideration and withdrawal of the Section 112 rejection.

RESPONSE TO SECTION 102 REJECTION

Claims 1-5, 9-20 and 24-27 stand rejected under 35 U.S.C. 102(b) for being anticipated by Weber, WO 94/02645. In particular, the Examiner contends that

> Weber (WO 94/02645, 3 Feb 1994) discloses a detection method for biopolymers in stained specimens. In the procedure of the invention, a fixative is used to fix cells. The fixative is a combination of precipitating fixatives (alcohols) and cross-linking fixatives (aldehydes). The fixative comprises ethanol, ethanol-acetic acid, methanol or methanol-acetone to provide good preservation of cellular morphology and preservation and accessibility of antigens and high hybridization efficiency (page 8, lines 8-29). Simultaneously, the fixative contains glutaraldehyde or formaldehyde which fixes the cellular components by cross-linking materials together (page 9, lines 24-26). In one embodiment, the solution contains guanidinium isothiocyanate, formamide, PEG, DTT, Ficoll/PVP, EDTA, salmon sperm DNA, Tris-acetate and Triton X-100 (page 20, lines 3-10). The procedure allows simultaneous detection of different substances (mRNAs, DNAs and proteins) within the same cells (page 19, lines 13-15).

Applicants respectfully disagree with the Examiner's rejection.

Weber purports to describe methods which may be used to detect microorganisms. *See* page 5, line 15 and following. The methods "detect and quantitate the presence of cellular genes from previously stained mammalian specimens." *See* page 6, lines 3-5, emphases added. "The cells and tissue specimens used in" the Weber method "may have been stained by any of the commonly used stains in cytology or histology." *See* page 6, lines 23-25. Thus, the methods described in Weber are not directed to the analysis of cellular morphology. As stated in the abstract: "The present invention provides methods to detect the presence of a biopolymer in a previously stained specimen using novel *in situ* hybridization techniques." Therefore, any medium described in Weber is not a medium considered capable of allowing both

direct cytological and direct molecular methods of analysis as Weber describes previously stained samples which are then examined by its methods.

To anticipate a claim, the reference must teach every element of the claim. "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628 (Fed. Cir. 1987). "The identical invention must be shown in as complete detail as is contained in the ... claim." *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236 9 (Fed. Cir. 1989).

Weber describes several different media. For example, a medium is described on page 7 under the heading "Cell Preparation for One-Step Method"

> This solution contains the following: optionally, a mild fixative, a chaotrope or other denaturing agent, a synthetic oligonucleotide probe (RNA or DNA probe which is prelabeled) and/or antibody proble, salts, detergents, buffers, and blocking agents.

Page 7, lines 26-29. The "mild fixative" is described further on page 8, which is the section of the specification the Examiner has referred to.

> The fixative may be a combination of precipitating fixatives (such as alcohols) and cross-linking fixatives (such as aldehydes), with the concentration of the cross-linking fixatives kept very low (less than 10%)." * * * Preferably, fixatives for use in the invention are selected from the group consisting of ethanol, ethanol-acetic acid, methanol, and methanol-acetone. Fixative most preferable for practicing the one-step procedure include 10-40% ethanol, 10-40% methanol, 10-40% acetone or combination thereof. These fixatives provide good preservation of cellular morphology and preservation and accessibility of antigens, and hybridization efficiency. Typically, the solution contains 1-40% ethanol, and 5% formalin.

Page 8, lines 8-29. However, there is no description of a water based medium that comprises a preservative, a cross-linking agent and an anti-degradation agent as described in applicants' claims. In particular, there is no description of an anti-degradation agent.

The Examiner has also referred to a medium described at page 9 under the heading "Fixation of Cells/Tissues in the Two-Step Method." It appear that these fixatives are used independently of other media described in the Weber application. The fixative described in this second formulation in Weber is as follows.

> * * * A fixative may be selected from the group consisting of any precipitating agent or cross-linking agent used alone or in combination, and may be aqueous or non-aqueous. The fixative may be selected from the group consisting of formaldehyde solutions, alcohols, salt solutions, mercuric chloride sodium chloride, sodium sulfate, potassium dichromate, potassium phosphate, ammonium bromide, calcium chloride, sodium acetate, lithium chloride, cesium acetate, calcium or magnesium acetate, potassium nitrate, potassium dichromate, sodium chromate, potassium iodide, sodium iodate, sodium thiosulfate, picric acid, acetic acid, paraformaldehyde, sodium hydroxide, acetones, chloroform, glycerin and thymol.
>
> * * * Preferably, fixatives for use in the invention are selected from the group consisting of ethanol, ethanol-acetic acid, methanol, and methanol-acetone which fixatives afford the highest hybridization efficiency with good preservation of cellular morphology.
>
> * * * Examples of cross-linking agents include paraformaldehyde, formaldehyde, dimenthylsilserimidate and ethyldimethylamino-propylcarbodimide.

Page 9, line 13 through page 10, line 4.

A third medium is described at page 11 under the heading "Hybridization Solution Components." Although the hybridization cocktail is described as possibly containing "precipitating and/or cross-linking fixatives," there is no mention of any media containing an anti-degradation agent. The hybridization solution is described as follows.

> The hybridization solution may typically comprise a chaotropic denaturing agent, a buffer, a pore-forming agent, a hybrid stabilizing agent. The chaotropic denaturing agents include formamide, urea, thiocyanate, guanidine, trichloroacetate, tetramethylamine, perchlorate, and sodium iodide. Any buffer which maintains pH at least between 7.0 and 8.0 may be utilized.

> The pore-forming agent is, for instance, a detergent such as Brij 35, Brij 58, sodium dodecyl sulfate, Tween, CHAPS or Triton X-100. * * *

A preferred embodiment of the hybridization solution is described at page 16. However, there is no mention of a preservative, nor a cross-linking agent, nor an anti-degradation agent as claimed in applicants' invention. The preferred embodiment of the Weber application is described as follows.

> In a preferred embodiment, the hybridization solution of the one-step *in situ* method consists of 25% formamide, 5X SSC, 15X Ficoll/PVP, .4 M guanidinium isothiocyanate, about 50 mM sodium phosphate (pH 7.4), 50 mM DTT, about 1 mg/ml salmon sperm DNA, 5% Triton X-100, 50 mM EDTA and 21% PEG.

A kit is also described in Weber at page 20. The kit is reported to include a solution containing a fixation/hybridization cocktail. It is to this solution that the Examiner refers. Weber describes the solution as follows.

> A solution containing a fixation/hybridization cocktail and one or more labeled probes. Preferably, this solution will contain 50 mM guanidinium isothiocyanate, 25-40% formamide, 21% PEG, 0.4 M DTT, 15X Ficoll/PVP, 50 mM EDTA, 1 mg/ml salmon sperm DNA, 50 mM Tris-acetate (pH 7-8), about 5% Triton X-100, and about .06 μg/μl of a synthetic oligonucleotide probe directly labeled with a reporter molecule.

Page 20, lines 4-9. However, the preferred solution described does not describe a preservative, nor a cross-linking agent nor an anti-degradation agent. In fact, nowhere does Weber describe a water based medium that comprises a preservative, a cross-linking agent and an antidegradation agent.

If, for the sake of argument, the passage at page 20, lines 4-9 is alleged to imply mixing a fixative with the described solution, this does not meet the requirements for a finding of anticipation. "A claim is anticipated only if each and every element as set forth in the claim is

found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628 (Fed. Cir. 1987). "The identical invention must be shown in as complete detail as is contained in the ... claim." *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236 9 (Fed. Cir. 1989). Thus, merely an implication does not rise to the level of anticipation. Second, even the allegedly implied solution does not teach one of skill in the art how to make and use a water based medium that comprises a preservative, a cross-linking agent and an antidegradation agent which allows for both direct cytological and direct molecular analysis. The implied solution would contain so many different components that one skilled in the art would not be taught that the components described in applicants' claims are the important components for carrying out applicants invention.

Finally, the Examiner refers to page 19, lines 13-15 of Weber as indicating that the "procedure allows simultaneous detection of different substance (mRNAs, DNAs and proteins) within the same cells." However, applicants claims describe methods and media that allow both molecular and cytological analysis. This is nowhere described in Weber.

In conclusion, the Examiner has referred to the fixative described on page 9 of Weber as "a combination of precipitating fixatives (alcohols) and cross-linking fixatives (aldehydes)." The Examiner also refers a specific embodiment, a fixation/hybridization cocktail described on page 20 of Weber, which does not describe a preservative or a cross-linking agent. Thus, neither of these media describe "each and every element as set forth in [any] claim," i.e., a water based medium that comprises a preservative, a cross-linking agent and an antidegradation agent. Although the Examiner refers to the Weber method as allowing "simultaneous detection of different substances" Weber does not describe methods or media which allow for direct molecular and direct cytological analysis. In fact, Weber describes that samples are first stored

in a transport medium. *See* page 21, lines 4-5. The sample is then stained, treated with a subsequent medium and examined by *in situ* hybridization. Thus, it is clear that none of the media described in Weber meet "each and every element as set forth in [any] claim."

In light of the arguments presented above, applicants respectfully request reconsideration and withdrawal of the Section 102 rejection.

RESPONSE TO SECTION 103 REJECTION

Claims 1-20 and 24-27 stand rejected under 35 U.S.C. 103(a) for being unpatentable over Weber in view of Hurley. In particular, the Examiner refers to Weber's description of a fixative containing an alcohol and an aldehyde on pages 8 and 9. The Examiner also refers to Weber's description of a specific embodiment which does not contain a preservative nor a cross-linking agent. As described above, Weber does not describe "each and every element as set forth in [any] claim."

The Examiner refers to Hurley as describing "a cell preservative solution containing an alcohol selected from the group consisting of ethanol and methanol, a chelating agent selected from the group consisting of EDTA and its salts, and a buffering agent to maintain the pH at from about 4 to about 7 for the duration of the preservation." However, Hurley does not rectify the deficiencies of Weber.

The Examiner contends that "[i]t would have been obvious to one of ordinary skill in the art at the time the invention was made to perform the method of analysis of cells or tissues using a medium containing a buffer, a preservative, a cross-linking agent and an anti-degradation agent as per the teachings of Weber and to further specify an effective pH range of about 4 because preserving the pH at about 4 for the duration of the preservation would result in effective preservation of the specimen, as per the teachings of Hurley." However, as described

above, Weber does not describe a medium containing a buffer, a preservative, a cross-linking agent and an anti-degradation agent as claimed by applicant. The description in Hurley of "an effective pH range of about 4" does not rectify the deficiencies of Weber. The combination of Weber and Hurley fails to make obvious applicants' claims. Therefore, in light of the arguments presented above, applicants respectfully request reconsideration and withdrawal of the Section 103 rejection.

Claims 21-23 stand rejected under 35 U.S.C. §103(a) for being unpatentable over Weber in view of Wainwright (U.S. Patent No. 5,370,128). Wainwright is relied on for its alleged disclosure of "a pap brush and pap unit container system for preserving a cell sample." Weber is referred to for its alleged disclosure of a medium containing a buffer, a preservative, a cross-linking agent and an anti-degradation agent.

However, as described above, Weber does not disclose each and every element as set forth in [any] claim." Wainwright's alleged disclosure of a pap brush and pap unit container system for preserving a cell sample does not rectify the deficiencies of Weber. Therefore, applicants respectfully request reconsideration and withdrawal of the Section 103 rejection.

AUTHORIZATION

No additional fee is believed to be necessary. The Commissioner is hereby authorized to charge any additional fees which may be required for this amendment, or credit any overpayment to Deposit Account No. 13-4500, Order No. 2629-4005US1.

In the event that an extension of time is required, or which may be required in addition to that requested in a petition and for an extension of time, the Commissioner is requested to grant a petition for that extension of time which is required to make this response timely and is hereby authorized to charge any fee for such an extension of time or credit any overpayment for an

extension of time to Deposit Account No. 13-4500, Order No. 2629-4005US1. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

Respectfully submitted,

MORGAN & FINNEGAN, L.L.P.

Dated: June 13, 2000

By: Darryl H Steensma
Darryl H. Steensma
Registration No. 43,155

Mailing Address:

MORGAN & FINNEGAN, L.L.P.
345 Park Avenue
New York, New York 10154
(212) 758-4800
(212) 751-6849 Facsimile